10/511685

Rec'd PCT/PTO 19 OCT 2004

PCT/GB 2003/001689

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

PRIORITY DOCUMENT

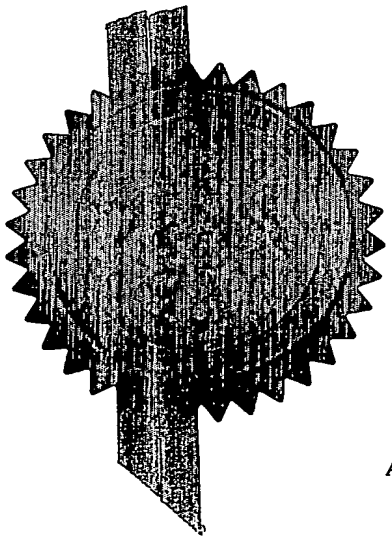
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.



Signed P. Mahoney

Dated 13 May 2003

22APR02 E712559-1 D00027
P01/7700 0.00-0209007.4

Patents Form 1/77

Patents Act 1977
(Rule 16)

The Patent Office

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

19 APR 2002

The Patent Office
Cardiff Road
Newport
Gwent NP9 1RH

1.	**Your reference**	444.77693/000
2.	**Patent application number** *(The Patent Office will fill in this part)*	0209007.4
3.	**Full name, address and postcode of the or of each applicant** *(underline all surnames)*	Norferm DA Vasabomen PO Box 8005 N-4003 Stavanger Norway
	Patents ADP number *(if you know it)*	7836265002
	If the applicant is a corporate body, give country/state of incorporation	Norway
4.	**Title of the invention**	Product
5.	**Name of your agent** *(if you have one)*	Frank B. Dehn & Co.
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	179 Queen Victoria Street London EC4V 4EL
	Patents ADP number *(if you know it)*	166001

6.	**If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and** *(if you know it)* **the or each application number**	**Country**	**Priority application number** *(if you know it)*	**Date of filing** *(day / month / year)*

7.	**If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application**	**Number of earlier application**	**Date of filing** *(day / month / year)*

8.	**Is a statement of inventorship and of right to grant of a patent required in support of this request?** *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *See note (d))*	Yes

Patents Form 1/77

9) Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	0
Description	9
Claim(s)	1
Abstract	-
Drawing(s)	-

10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination and search *(Patents Form 9/77)*	-
Request for substantive examination *(Patents Form 10/77)*	-
Any other documents *(please specify)*	Copies of GB0203306.6 and 0203307.4

11. I/We request the grant of a patent on the basis of this application.

Signature Frank B Dehn & Co Date 19th April 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Julian Cockbain
020 7206 0600

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*
b) *Write your answers in capital letters using black ink or you may type them.*
c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s) of the form. Any continuation sheet should be attached to this form.*
d) *If you have answered 'Yes', Patents Form 7/77 will need to be filed.*
e) *Once you have filled in the form you must remember to sign and date it.*
f) *For details of the fee and ways to pay please contact the Patent Office.*

77693000.617

Product

The present invention relates to the use as a microorganism growth substrate of a bacterial biomass, in particular a biomass, herein termed a "bacterial extract", deriving at least in part from a bacterial culture comprising a methanotrophic bacterium.

Microorganisms are frequently grown on commercial and laboratory scales, for example to produce desired substances from bacterial strains which either naturally produce such substances or have been genetically modified so as to produce such substances, or so as to determine the nature of a bacterial contamination, etc. For these purposes the microorganisms require nutrients and in this regard it is conventional to use yeast, meat or plant extracts which are widely available commercially, e.g. MRS (De Mann, Rogosa and Sharpe), PCA (Plate Count Agar), VRBD (Violet Red Bile Dextrose Agar), YM Agar (Yeast Mould Agar), Baird-Parker Agar Base, VRB Agar (Violet Red Bile Agar), XLD Agar (Xylose Lysine Deoxycholate Agar), CASO (Casein-peptone Soybean-peptone), TSB (Tryptic Soy Broth) and NB (Nutrient Broth). Yeast extract growth substrates (contained for example in MRS, VRBD, VRB Agar, PCA, Baird-Parker Agar Base, and XLD Agar) are available commercially from Merck and Difco among others. Such yeast extracts are commonly produced using biomass from yeast cultures which has been allowed to autolyse, i.e. enzymes naturally occurring within the yeast cells act to break down the cells after cell death. Autolysis of yeast is generally slow and several days may be needed before a suitable degree of digestion is achieved. Accordingly, additives which act as autolysis initiators or stimulators, e.g. thiol agents, are generally used to accelerate the autolysis process. The use of such additives of course adds to the costs of commercial

production of yeast autolysates. To the resulting autolysates, extra nutrients may be added to optimize cell growth for particular microorganisms and indeed library deposits of microorganisms will generally specify which growth medium is most suitable for the deposited organism.

Since different microorganisms have different nutritional needs there is of course an ongoing need for alternative and improved microorganism growth media, particularly for growth media effective for growing those microorganisms which are challenging to grow in vitro (e.g. lactobacilli) and for "broad spectrum" growth media which may be suitable for use with unknown microorganisms.

We have now surprisingly found that particularly effective microorganism growth media may be produced using the biomass harvested from a culture medium comprising methanotrophic bacteria, e.g. biomass produced as described in WO 01/60974.

Viewed from one aspect therefore the present invention provides the use of a sterile nutrient composition derived from the biomass of a culture of bacteria including methanotrophic bacteria, and optionally containing further nutrients, as a microorganism growth medium.

The bacterial culture used to produce the biomass is preferably at least 50%, more preferably at least 60%, especially at least 70%, in particular at least 75%, e.g. 75 to 95%, more particularly 75 to 80%, by weight methanotrophic bacteria (relative to the total bacterial weight).

Viewed from a further aspect the invention provides a method of culturing microorganisms which comprises bringing together a microorganism and a growth medium therefor, characterised in that said growth medium is or is prepared from a sterile nutrient composition derived from the biomass of a culture of bacteria including

methanotrophic bacteria, optionally with the addition of further nutrients.

Viewed from a yet further aspect the invention provides a microorganism growth substrate comprising a sterile nutrient composition derived from the biomass of a culture of bacteria including methanotrophic bacteria, further containing at least one sterile nutrient, and optionally containing a diluent.

The biomass from which the growth medium or substrate is prepared is preferably biomass generated from at least one species of methanotrophic bacteria and at least one species of heterotrophic bacteria, preferably grown in the same culture medium, e.g. using a loop reactor provided with methane, oxygen, ammonia, and mineral feeds. Suitable combinations of bacteria for generating the biomass are described for example in WO 01/60974 the contents of which are incorporated by reference. One particularly suitable combination is Methylococcus capsulatus (Bath) (strain NCIMB 11132), Ralstonia sp. DB3 (strain NCIMB 13287), Aneurinibacillus sp. DB4 (strain NCIMB 13288) and Brevibacillus agri DB5 (strain NCIMB 13289).

The biomass from the bacterial culture may be used directly (although generally after dewatering and sterilization) or it may first be processed to break down the bacterial cells, e.g. by homogenization, hydrolysis or autolysis. Such treatments are described in WO01/60974 and British Patent Applications Nos. 0203306.6 and 0203307.4 filed 12 February 2002 which are also incorporated herein by reference. (Copies of these two British Patent Applications are also filed herewith.) While homogenizate, hydrolysate and autolysate, especially dried homogenizate and more especially dried autolysate, of the bacterial biomass are the preferred materials for the preparation of microorganism growth media according to the invention, precursor materials obtained by filtration (e.g. ultra

filtration) of the homogenized, autolysed or hydrolysed biomass, i.e. the liquid filtrate itself and the retentate, may also be used. Most preferred however is the dried autolysate.

The microorganism growth medium may be the bacterial biomass product itself or a composition containing the biomass product and further constituents, e.g. a liquid or non-liquid carrier or diluent (such as water, gel (e.g. agar gel), or a gellable liquid), and materials such as minerals, carbon sources (such as saccharides (e.g. mono, di, oligo and polysaccharides, especially mono and disaccharides)), nitrogen sources (e.g. nitrates, proteins or protein fragments, ammonium compounds, oligopeptides, amino acids (especially essential amino acids, e.g. tryptophan)), nucleic acids and nucleic acid fragments, lipids, etc. Particularly preferably the medium contains glucose and added nitrate and mineral salts (e.g. potassium, calcium, magnesium, sodium, molybdenum, iron, zinc, boron, cobalt, manganese and nickel compounds), especially glucose. The composition as provided may be a sterile solid (e.g. particulate), a semi-solid or a liquid in ready to use or concentrate form. Especially preferably the composition as provided will be a sterile dry particulate concentrate transformable into a growth medium by the addition of water or an aqueous gelling agent composition.

Wherein the composition contains added glucose, this is preferably in a dry mass basis weight ratio of 5:1 to 1:5 (especially 2:1 to 1:2) relative to the biomass deriving component. Where the composition contains added nitrate and mineral salts those preferably in a weight ratio of 0.01:1 to 0.2:1 (especially 0.05:1 to 0.1:1) relative to the biomass deriving component. Where the composition as provided contains no added glucose and/or nitrate mineral salts, it is preferred that the preparation of the growth

medium involve addition of one or both such components in the weight ratios specified above.

The compositions of the invention are particularly suitable for use as growth substrates for heterotrophic microorganisms, especially heterotrophic algae, yeast or bacteria, in particular anaerobic bacteria such as lactobacilli, aerobic bacteria such as E. coli, and algae such as Crypthecodinium cohnii.

The invention will now be illustrated further with reference to the following non-limiting Examples.

EXAMPLE 1

Biomass Extracts

Methanotrophic and heterotrophic bacteria (Methylococus capsulatus (Bath) (strain NCIMB 11132) Ralstonia sp. DB3 (strain NCIMB 13287), Aneurinibacillus sp. DB4 (strain NCIMB 13288) and Brevibacillus agri DB5 (strain NCIMB 13289)) were cultivated as described in WO01/60974 and the resulting biomass harvested and treated as described in WO01/60974 to produce spray-dried homogenizate (hereinafter "BP Homogenizate"), as described in British Patent Application No. 0203306.6 to produce spray-dried hydrolysate (hereinafter "BP Hydrolysate"), and as described in British Patent Application No. 0203307.4 to produce an autolysate (hereinafter "BP Autolysate"). Where the post-autolysis ultrafiltration and evaporation steps in the production of BP Autolysate are omitted, the product is referred to herein as "BP Crude Autolysate". The product referred to as "BP Retentate" is an ultra-high-temperature treated biomass that was homogenized. The product referred to as "BP Permeate" corresponds essentially to the liquid by-product of the ultrafiltration step in the production of BP Homogenizate. Microorganism growth media were produced by adding BP Homogenizate, BP Crude Autolysate, BP Autolysate, BP Retentate and BP Permeate to

demineralized water at a concentration of 1g/L. These media were then used either directly or with the addition of 0.1 g/L glucose and/or 32.4 mL/L Nitrate Mineral Salt medium (NMS).

NMS comprises:

1.0g KNO_3
0.2g $CaCl_2.2H_2O$
1.0g $MgSO_4.7H_2O$
0.1 mL trace element solution*
0.1 mL sodium molybdate solution (5g/L $NaMoO_4.2H_2O$ in demineralized water)
0.1 mL EDTA solution (45g/L $FeNaEDTA.2H_2O$ in water)
water - to 1L
10 mL/L sterile phosphate buffer (35.6g $Na_2HPO_4.2H_2O$, 26.0g KH_2PO_4 and water to 1L) was added.

*6.4g $ZnSO_4.7H_2O$
150 mg H_3BO_3
600 mg $CoSO_4.7H_2O$
130 mg $MnCl_2$
100 mg $NiCl_2.6H_2O$
demineralized water - to 1L

All media were autoclaved before use.

An agar-based microorganism growth medium was also prepared containing:

32.2 g/L BP Autolysate
20.0 g/L glucose
34 mL/L NMS medium
14.0 g/L agar
demineralized water to 1L

This was autoclaved before use.

EXAMPLE 2

Microorganism Growth Tests

Aerobic and anaerobic, Gram positive and Gram negative bacteria were grown in a shake flask using the liquid growth media of Example 1 and, as controls, growth media recommended for the bacterial strains. The optical density of the cultures was monitored as an indicator of the obtained bacterial growth (i.e. the "plateau" or stationary phase with the highest number of cells). The results are set out in Table 1 below.

Table 1

Bacterium	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Lactobacillus plantarum*	*Escherichia coli*
Characteristics	G(-), aerob	G(+), aerob	G(+), anaerob	G(-), aerob
Control Substrate	CASO	NB	MRS	TSB
BP Homogenisate	+	+++	+++	+
BP Autolysate	+++	+++	+++	+++
BP Crude autolysate	---	+++	+++	+++
BP Retentate	+	+++	+++	+++
BP Permeate	+	+	+++	---

--- : No combinations of the BP derivative were better than control substrate.

+ : Some combinations of the BP derivative were as good as the control substrate.

+++ : Some combinations of the BP derivative were clearly better than control substrate.

For E. coli, BP Autolysate with added glucose clearly provided the best growth. For L. plantarum, all combinations of BP Autolysate clearly gave the best growth. For P. aeruginosa, all combinations of BP autolysate gave the best growth, in particular BP

Autolysate with added glucose. For B. subtilis, BP Autolysate with added glucose and both glucose and NMS added clearly gave the best growth.

EXAMPLE 3

Viability of Unknown Bacteria

Agar gels (PCA, MRS-agar, and BP Autolysate with agar(Example 1) pH 6.0 and 7.1) were spread with unknown microorganisms taken from a sample of chopped meat. The cultures were incubated for 72 hours at 25°C and the total plate count was recorded. For BP Autolysate, log (CFU/g) was between 5 and about 6.6 while for MRS it was less than 1. For PCA log(CFU/g) was about 6.7, i.e. barely higher.

EXAMPLE 4

Lactobacillus Viability

The lactobacillus strains L. casei ssp. rhamnosus (strain ATCC 7469), L. delbruekii ssp. lactis (strain ATCC 7830), L. fermentum (strain CCUG 30138), L. gasseri (strain ATCC 19992), and L. plantarum (strain ATCC 8014) were grown under aerobic conditions on MRS agar and BP Autolysate agar (Example 1). In all cases the viability, measured as log(CFU/mL), was the same or greater for BP Autolysate. This was most pronounced for L. delbruekii. The same strains were also grown on these media under anaerobic conditions and again viability was the same or better in all cases for BP Autolysate.

EXAMPLE 5

Production of Polyunsaturated Fatty Acids

Crypthecodinium cohnii (Seligo) Javornicky (strain ATCC 30772) was grown on a culture medium comprising 9g/L

glucose, 25g/L sea salt and 2g/L of either yeast extract (YE) or BP Autolysate in demineralized water. After two days of incubation, the cells were harvested and the total fatty acid, cell dry weight (CDW) and 22:6 (docosahexaenoic acid) contents were determined. The results are set out in Table 2 below.

Table 2

Culture Medium	CDW (g/L)	Lipid (%)	Lipid (g/L)	22:6 (%)	22:6 (g/L)
YE	3.2	12.0	0.39	36.1	0.139
BP Autolysate	4.2	7.9	0.33	40.9	0.135

Table 2 shows that the CDW and the percentage of the polyunsaturated fatty acid 22:6 was higher when BP Autolysate was used.

Claims:

1. The use of a sterile nutrient composition derived from the biomass of a culture of bacteria including methanotrophic bacteria, and optionally containing further nutrients, as a microorganism growth medium.

2. A method of culturing microorganisms which comprises bringing together a microorganism and a growth medium therefor, characterised in that said growth medium is or is prepared from a sterile nutrient composition derived from the biomass of a culture of bacteria including methanotrophic bacteria, optionally with the addition of further nutrients.

3. A microorganism growth substrate comprising a sterile nutrient composition derived from the biomass of a culture of bacteria including methanotrophic bacteria, further containing at least one sterile nutrient, and optionally containing a diluent.